American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)

ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

The Prevalence of Listeria monocytogenes In Placental Tissue from Abortion and Fetal Death at Mother and Child's Sri Ratu Hospital, Medan, Indonesia

Mangatas Silaen[a]*, Ety Yerizel[b], Sumaryati Syukur[c], Endang Purwati[d]

[a]*Department of Obstetric and Ginecology, Faculty of Medicine, University of Prima Indonesia, Medan, Indonesia*

[b]*Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, Indonesia*

[c]*Laboratory of Biochemistry and Biotechnology,Department of Chemistry, Faculty of Mathematics and Natural Science, Padang, Indonesia*

[d]*Head of Animal Production Technology Laboratory Faculty of Animal Farm Field of Probiotics and Halal Food (Bioteknolgi) Andalas University , Padang , Indonesia*

[a]*Email: mangatassilaenobgin@gmail.com*

[b]*Email: ety_yerizel@yahoo.co.id*

[c]*Email: sumaryatisyukur_unand@yahoo.co.id*

[d]*Email: purwati17@yahoo.co.id*

Abstract

For the identification of pathogenic bacteria *Listeria monocytogenes* (*L.monocytogenes*) in vitro, the method of examination that currently most reliable is the growth in the culture medium, followed by isolation and identification of biochemical and serological. Total of 98 samples were collected from placental tissue curettage resulted from abortion at Mother and Child's Sri Ratu Hospital Medan, North Sumatra, Indonesia. Curettage was undertaken in accordance with the working procedures issued by the research ethics committee of the Faculty of Medicine, University of Andalas Padang, West Sumatra, with a qualifying ethic examine, No: 190 / KEP / FK / 2015. The media we use to isolate and identification are Listeria Enrichment Broth (Biolife Italia) and *Agar Listeria Ottaviani Agosti (ALOA)* (*Biolife Italia)*. All the tissues examined were found: *Listeria* (9%) include, *L. monocytogenes* isolates (90%), and *L.innocua* (10%). We conclude that *L.monocytogenes* has been found in placental tissue in less than 20 weeks of abortion in Mother and Child's Sri Ratu Hospital Medan, North Sumatra, Indonesia.

---

* Corresponding author.

The aim of this study was to investigate other causes of abortion and fetal death in utero less than 20 weeks and whether *L.monocytogenes* has become another cause of abortion.

Keywords: Abortion; Placental tissue culture; *L.monocytogenes.*

1. Introduction

Food safety has become an important global issue in the International free trade, this will have an impact on the public health implications of the world [1]. *L. monocytogenes* bacterial pathogens was reported cause outbreaks of illness associated with different types of food [2].Various types of food that have been studied such as meat, milk and dairy products are taken from various countries contain bacteria [3,4,5,6], but the isolation of the bacteria from the first trimester abortion's tissue is rarely reported.

L. monocytogenes is considered a dangerous agent in the food industry, because of its ability to grow and multiply at low temperature, gas or food stored in the refrigerator. It is because the pathogens can grow at 4 ^{0}C to 37 ^{0} C and form biofilms [7,8]. *L. monocytogenes* also can survive in extreme pH and resistance to heavy metals or other cleaning agents [9].

Listeria monocytogenes is Gram-positive rod-shaped bacteria, nonsporulating, facultative intracellular. It is a food pathogen that can cause various diseases such as abortion, missed abortion or death after birth, meningitis and meningoencephalitis. Individuals who are most at risk are pregnant women, newborns, elderly and those who suffer immunocompromised [10,11,12,13]. This disease has been reported in women with bad obstetric history and recurrent abortion from various countries, including India [14,15,16].

Listeria monocytogenes is also found as many as more than 10% in normal individuals, and are usually found in the intestines of healthy people [17]. Unlike common foodborne pathogen, such as *Salmonella*, which rarely cause mortality. Listeriosis is actually associated with high case mortality rate ± 30%, [18].

One data obtained and reported 178 cases found 36 cases of mothers were suffering listeriosis (20.2%) and had a spontaneous abortion. In the remaining 142 cases, 97 neonates (68.3%) were born with infection. 97 neonatal cases it was reported that 23 neonatal (24.5%) died of infection, whereas 12 (12.8%) had serious long-term complications [19]. Therefore it is a big concern for obstetricians to prevent, diagnosis and giving therapy to pregnant women. In addition *Listeria monocytogenes* bacteria can also be found in healthy individuals [20].

The current microbiological methods were undertaken to identify *L.monocytogenes* in culture media followed the process of isolation, biochemical and serological identification. However, this method is laborious and time consuming which requires a minimum of five days to identify the pathogen *L.monocytogenes* [21] and about ten days to identify *L. monocytogenes* with confirmatory tests [22].

Listeria are small Gram-positive bacilli, ubiquitous, non-spore forming, facultative anaerobic bacteria that grow between -2 and 50 ° C, with optimal growth between 30 and 37 ° C. [23,24] Officially the *Listeria* genus includes six species: *Listeria monocytogenes, Listeria ivanovii, Listeria innocua, Listeria seeligeri, welshimeri*

Listeria, *Listeria grayi* [25]. Two other species, *Listeria marthii* (close to *L. monocytogenes* and *L. innocua*) and *Listeria rocourtiae* (close to *L. grayi*) have recently been described [26,27]; but have not yet been introduced into the official classification [25]. Among the reviews, *L. monocytogenes* is the most reported as pathogenic for humans [28]. However, some cases of listeriosis have been also attributed to *L. ivanovii* [29,30,31]. *L. innocua* [32] and *L. seeligeri* [33].

2. Methods and Materials

This research was conducted in Biochemistry Laboratory Faculty of Mathematics and Natural Sciences and Biotechnology Laboratory Faculty of Animal Husbandry Andalas University of Padang from 1st July 2015 to 1st January 2017.

The type of data we used in this study are primary and secondary data. Primary data was obtained from the laboratory results and secondary data was obtained from the literature study and related research journals that supported this research

The data was collected with documentation study method. This study is a technique by collecting and analyzing documents, either written document, picture or electronic. Documents that have been obtained then was analyzed, compared and combined to form a systematic, coherent and intact study result.

The equipment used in this research includes autoclave, laminar air flow cabinet, petri dish, measuring pipette, test tube, reaction tube shelf, erlenmeyer, ose needle, incubator, measuring cup, beaker glass, glass object, microscope, dropper drop, magnetic stirrer and hot plate, colony counter, water bath, vortex (Heidolph), eppendorf 0.5 1.5 and 2 ml tubes, falcon tubes, micro pipettes, micro tip, spreader, centrifuge (Sartorius Sigma), biodoc analyze (Biometra), electrophoresis (Mupid Exu), thermocycler (Biometra) spatula, oven, desiccator, shaker, filter paper, analytical scales, label paper, paper wrap, aluminum foil and other tools.

The samples we used for this research is curettage placenta from pregnant women of 20 weeks diagnosed with ultrasound who have fetal or abortal tissue deaths, CHROMagar, and fraser broth (Oxoid, Basingstoke, UK).

3. Procedures

3.1. Listeria Bacteria Culture

We used two-step enrichment method of 1 gram of placenta added 10 ml of Fraser broth (Oxoid, Basingstoke, England) then homogenized with stomaker (Lab blender 400, Seward Medical, London, UK)) and incubated for 24 hours at 30 ° C. A circle of first enriched broth cultures was reproduced in CHROMagar (CHROMagar Listeria cc.to Ottaviani & Agosti Listeria ALOA., Paris, France). L.monocytogenes grow as a purple colony surrounded by halo circles (typical colonies). If it is not a Listeria monocytogenes, the bacteria will show a blue-green colony with no circles and a hazy halo. If no typical colonies are present after 24 hours of incubation or if Listeria growth does not occur then incubation was repeated for 18-24 hours later. If no typical colonies growth, the sample may be considered as L.monocytogenes-free. If a typical colony grows during the second period of incubation we make sure these colonies as described above.

4. Results

4.1. The Characteristic of Pregnant Women with Abortion or Fetal Death at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017

Total of 98 patients who received curettage at Women at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017 who was diagnosed with abortion or fetal death in utero through ultrasound with gestational age below 20 weeks. The results are in Table 1 below.

Table 1: Characteristics of pregnant women with abortion and fetal death before 20 weeks' gestation

No.	Category		Frequency	Percentage (%)
1.	Age	< 19 years	15	15,3
		20 – 35 years	63	64,3
		> 36 years	20	20,4
		Total	98	100
2.	Old pregnancy	0 – 12 weeks	45	45,9
		13 – 20 week	53	54,1
		Total	98	100
3.	Number of pregnancies	1 – 3	82	83,7
		> 4	16	16,3
		Total	98	100
4.	Number of abortions	1 – 3	95	97
		> 3	3	3
		Total	98	100
5.	Residen	City	82	83,7
		Village	14	16,3
		Total	98	100

From Table 1 data above, can be concluded that: the highest percentage of mother having an abortion who have done curettage with cause of growth disturbance and death of fetus is between age of 20 - 35 (64,3%). It is not much different from Anggun's research result [34] that reported 67% of mothers had abortion in reproductive age between 20-35 years.

The most frequent of miscarriage is 13 - 20 weeks' (54,1%) gestational age but not significantly different from 0 - 12 weeks' (45.9%). 1 of 6 pregnancies (17%) end in miscarriage and most often between 6 - 10 weeks of pregnancy [35].

The highest pregnancies frequency in the first to third pregnancy is 97% [36]. Based on Winkjosastro theory, the incidence rate of pregnant women who have abortion is more likely to occur in multiparity. This is because the

multiparity has been too often fertilized cause the uterus weakens [37] .

Residential factors is more common occur in pregnant woman abortion who live in the city is 83.7% this is because of many import foods in big cities.

4.2. The Prevalence of Placenta Containing L.monocytogenes from Curettage at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017

Total of 98 patients who done curettage at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017 who diagnosed with abortion or fetal death in utero through ultrasound with gestational age below 20 weeks. The results are in Table 2 below.

Table 2: The prevalence of placenta containing *L.monocytogenes*

No	Number	Percentages (%)
1. Found *Listeria*	9	9
2. Not found *Listeria*	79	81
Total	98	100

From Table 2 above can be concluded that: there are 9 isolated placenta containing *Listeria* bacteria (9%), this result is quite high compared to the study [38]. He reported the results of his study from 305 samples from blood, urine, placental, feces, and vaginal secretions collected from 61 patients with spontaneous abortion, found 10 isolates resembling *listeria* species and from 10 isolates, 4 are identified as *L.Monocytogenes*, which isolates 3 and 4 are found from medium placental, in other isolates found as *L. Seeligeri* and *L. welshimeri*. From the conclusion found 14.8% in the placenta of spontaneous abortus found *listeria* species and as many as *L.monocytogenes* 3.3%. Other researchers found CHROMagar's sensitivity to *L.monocytogenes* growth was 96.9% and 99.1% was found *L. monocytogenes* that detected in contaminated foods [39,40].

Listeriosis disease has also been reported which recurrent abortions are found in women with poor obstetric history and Indian [11,12,13,14,15,16].

4.3. Types of Listeria from Placenta Tissue Culture from Abortion at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017

Total of 98 patients conducted curettage at Mother and Child's Sri Ratu Hospital Medan from July 2015 to January 2017 who diagnosed with abortion or fetal death in utero through ultrasound with gestational age below 20 weeks. The results are in Table 3 below.

Table 3: Placental tissue culture results who diagnosed with abortion or fetal death in utero through ultrasound with gestational age below 20 weeks

No code Sample	Result	*L.monocytogenes*	*L.innocua*	Number	Presentase (%)
1,3,6,12,18,22,40,69	+	+		8	8
32	+		+	1	1
2,4,5,7,8,9,10,11,13,14,15 ,16,17,19,20,21,23,24,25, 26,27,28,29,30,31,33,34,3 5,36,37,38,39,41,42,43,44 ,45,46,47,48,49,50,51,52, 53,54,55,56,57,58,59,60,6 1,62,63,64,65,66,67,68,60 ,71,72,73,74,75,76,77,78, 79,80,81,82,83,84,85,86,8 7,88,89,90,91,92,93,94,95 ,96,97,98.	_	_	_	89	91
Total			98	100	

From Table 3. above can be concluded that: 98 samples by using CHROMagar found 9 isolates *listeria* (1,3,6,12,18,22,32,40,69), 8 is *L.monocytogenes* (90%) and 1 is *L.inocua* (10%). This result is quite high compared from S. Kaur research which 14.8% was found in women with spontaneous abortion and *Listeria monocytogenes* species were found 3.3%.

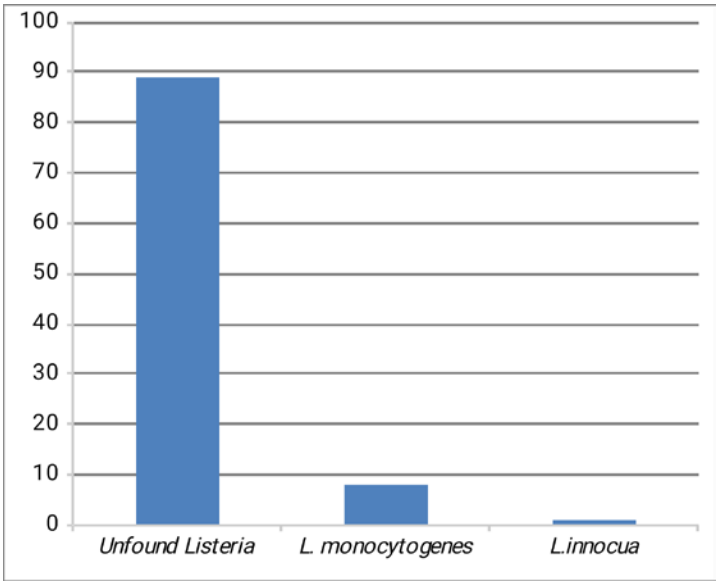


Figure 1: Placental tissue culture results

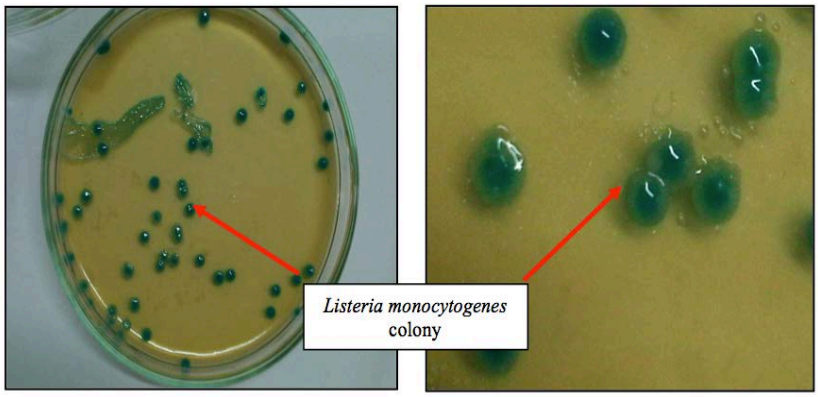


Figure 2: *L.monocytogenes* colony using Chrom agar

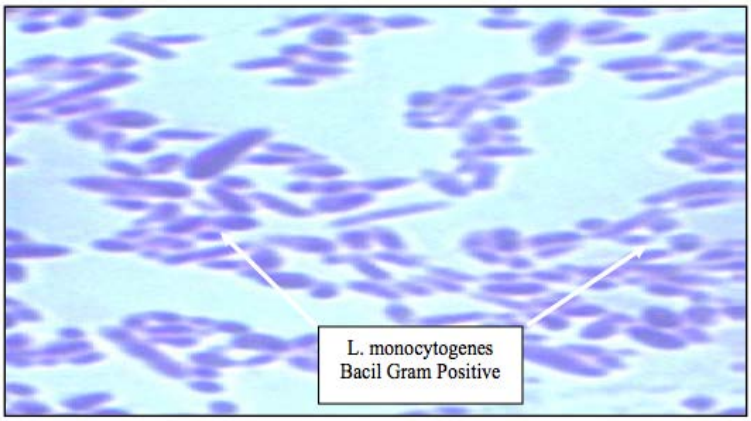


Figure 3: *Listeria monocytogenes staining*

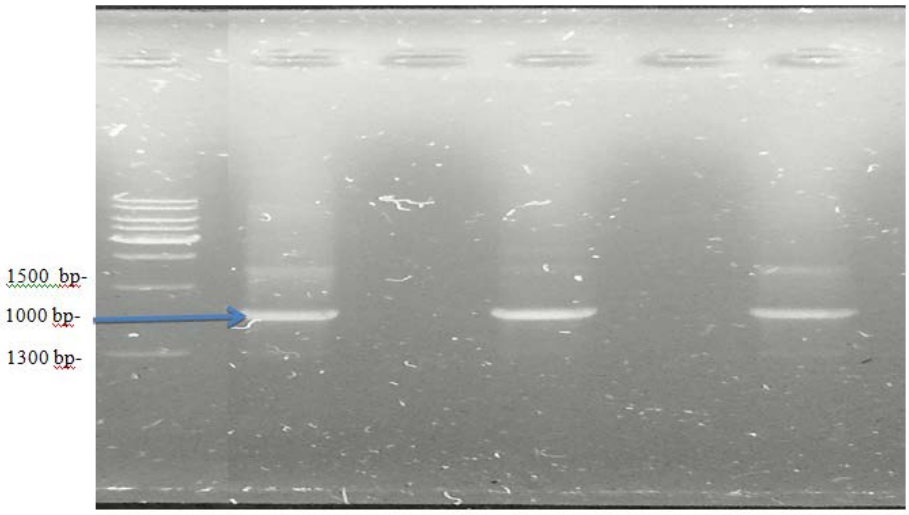
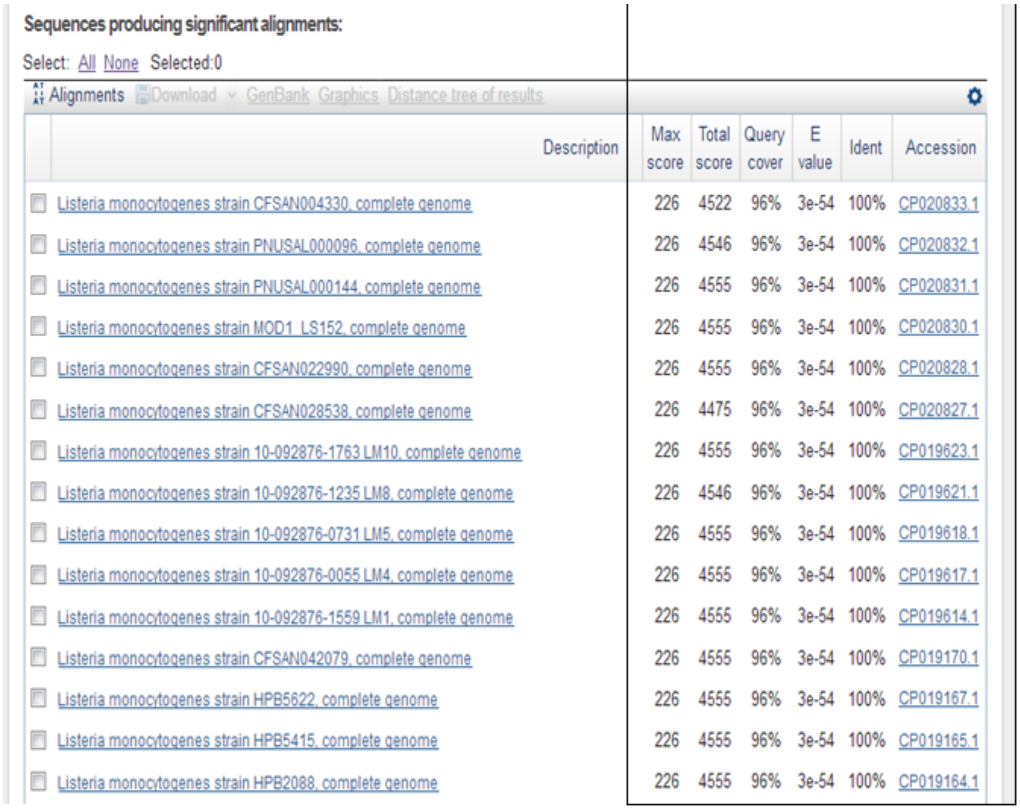


Figure 4: Isolated *Listeria monocytogenes* by PCR is CFSAN004330



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Listeria monocytogenes strain CFSAN004330, complete genome	226	4522	96%	3e-54	100%	CP020833.1
Listeria monocytogenes strain PNUSAL000096, complete genome	226	4546	96%	3e-54	100%	CP020832.1
Listeria monocytogenes strain PNUSAL000144, complete genome	226	4555	96%	3e-54	100%	CP020831.1
Listeria monocytogenes strain MOD1_LS152, complete genome	226	4555	96%	3e-54	100%	CP020830.1
Listeria monocytogenes strain CFSAN022990, complete genome	226	4555	96%	3e-54	100%	CP020828.1
Listeria monocytogenes strain CFSAN028538, complete genome	226	4475	96%	3e-54	100%	CP020827.1
Listeria monocytogenes strain 10-092876-1763 LM10, complete genome	226	4555	96%	3e-54	100%	CP019623.1
Listeria monocytogenes strain 10-092876-1235 LM8, complete genome	226	4546	96%	3e-54	100%	CP019621.1
Listeria monocytogenes strain 10-092876-0731 LM5, complete genome	226	4555	96%	3e-54	100%	CP019618.1
Listeria monocytogenes strain 10-092876-0055 LM4, complete genome	226	4555	96%	3e-54	100%	CP019617.1
Listeria monocytogenes strain 10-092876-1559 LM1, complete genome	226	4555	96%	3e-54	100%	CP019614.1
Listeria monocytogenes strain CFSAN042079, complete genome	226	4555	96%	3e-54	100%	CP019170.1
Listeria monocytogenes strain HPB5622, complete genome	226	4555	96%	3e-54	100%	CP019167.1
Listeria monocytogenes strain HPB5415, complete genome	226	4555	96%	3e-54	100%	CP019165.1
Listeria monocytogenes strain HPB2088, complete genome	226	4555	96%	3e-54	100%	CP019164.1

Figure 5: Isolated Listeria monocytogenes by Sequences strain is CFSAN004330

5. Conclusion

Listeria monocytogenes have been found in placental tissue of pregnant women with abortion below 20 weeks of gestational ages, at Mother and Child's Sri Ratu Hospital Medan North Sumatra, Indonesia.

Acknowledgements

Thanks, SKIM KLASTER RISET Prof. drh. Hj. Endang PRN, MS., Ph.D for providing BIOTEK Laboratory for research No: 53/UN.16.17/PP.HGB/LPPM/2017

Reference

[1] Sudershan RV, Rao P, Polasa K. "Food safety research in India". Asian J Food AgInd, vol. 2, pp. 412-433, 2009.

[2] Swaminathan B B, Gerner-Smidt P. "The epidemiology of human listeriosis". Microbes Infect, vol. 9, pp. 1236-1243, 2007.

[3] Ryser ET, Marth EH. Listeria, Listeriosis and Food Safety. New York: Marcel Dekker, 1991.

[4] Jaradat ZW, Schutze GE, Bhunia AK. "Among the genetic homogeneity of Listeria monocytogenes strains from infected Patients and meat products from two geographic locations determined by phenotyping, ribotyping and PCR analysis of virulence genes". Int J Food Microbiol, vol. 76, pp. 1-10, 2002.

[5] AMA El-Malek, Ali SFH, Hassanein R, Mohamed MA, Elsayh KI. "Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of Listeria monocytogenes". Vet. World, vol. 3, pp. 353-359, 2010.

[6] Ikeh MAC, Obi SKC, Ezeasor DN, Ezeonu IM, Moneke AN. "Incidence and pathogenicity profile of Listeria sp. isolated from food and environmental samples in Nsukka, Nigeria". Afr J Biotechnol, vol. 9, pp. 4776-4782, 2010.

[7] Le Monnier A, Autret N, Join-Lambert OF, Jaubert F, Charbit A, et al. "ACTA is required for the crossing of the fetoplacental barrier by Listeria monocytogenes". Infect Immun, vol. 75, pp. 950-957, 2007.

[8] Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, et al. "Conjugated action of two species-Specific invasion proteins for fetoplacental listeriosis". Nature, vol. 455, pp. 1114-1118, 2008.

[9] Robbins JR, Skrzypczynska KM, Zeldovich VB, Kapidzic M, Bakardjiev AI. "Placental syncytiotrophoblast constitutes a major barrier to vertical transmission of Listeria monocytogenes". PLoS Pathog, vol. 10, pp. 1371, 2010.

[10] Low, J.C. and Donachie, W. "A review of Listeria monocytogenes and listeriosis". Vet J, vol. 153, pp. 9-29, 1997.

[11] Gomez-Mampaso, E., Mochales Baquero, F., de Rafael Nerpel, L., Michaux Oquinena, L. and Garcia-Villalba, A. "Listeriosis and fertility (author's transl)". Reproduccion, vol. 4, pp. 309-314, 1980.

[12] Lorentzen, U., Nyholm, H.C. and Moller-Hansen, K.J. "Listeriosis i-graviditet Tredje trimester". Ugeskr-Laeger, vol. 159, pp. 2716, 1997.

[13] Tridente, V., Cataldi, UM, Mossa, B., Morini, F., Bonessio, L.,et al. "Caso d 'infezione MATERNA e neonatale provocata da Listeria monocytogenes". Clin Ter, vol. 149, pp. 307-311, 1998.

[14] Aljicevic, M., Beslagic, E., Zvizdic, S., Hamzic, S. and Mahmutovic, S. "Listeria monocytogenes in women of reproductive age". Med Arh, vol. 59, pp. 297-298, 2005.

[15] Bhujwala, R.A. and Hingorani, V. "Perinatal listeriosis: a bacteriological and serological study". Indian J Med Res, vol. 63, pp. 1503-1508, 1975.

[16] Gupta, V., Gautam, V., Mehta, N., Kumari, I. and Joshi, RM. "Listeriosis in second trimester of pregnancy: case report from India". Jpn J Infect Dis, vol. 56, pp. 60-61, 2003.

[17] Anon. "Listeriosis". Center for Food Security and Public Health. College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011. Available at: http://www.cfsph.iastate.edu/Factsheets/pdfs/listeriosis.pdf, 2005 [Accessed on 20 November, 2006].

[18] Gouws PA, Liedemann I. "Evaluation of Diagnostic PCR for the Detection of Listeria monocytogenes in Food Products". Food Tech Biotech, vol. 43, pp. 201-205, 2005.

[19] Mylonakis E, Paliou M, Hohmann E, Calderwood S, Wing E. "Listeriosis during pregnancy: a case series and review of 222 cases". Medicine, vol. 81, pp. 260-269, 2002.

[20] VS Parihar, Barbuddhe SB, Danielsson-Tham ML, Tham W. "Isolation and characterizationof Listeria species from tropical seafoods". Food Cont, vol. 19, pp. 566-569, 2008.

[21] JA Khan, RS Rathore, Ahmad I, Khan S. Molecular Strategies: Detection of Foodborne. Bacterial Pathogens. In: Ahmad I, Ahmad F, Pitchel J, ed. Microbes and Microbial Technology. New York: Springer, 2011, pp. 189-206.

[22] Anon. "Reporting and Surveillance guidelines for listeriosis. Communicable. Disease Epidemiology, Office of Epidemiology, Washington Sate Department of Health, Shoreline, USA". Available at: http://www.doh.wa.gov/Notify/guidelines/listeriosis.html. 2002 [Accessed on 20 November, 2006]

[23] Bajard S, Rosso L, Fardel G, Flandrois JP. "The particular behavior of Listeria monocytogenes under sub-optimal conditions". Int J Food Microbiol, vol. 29, pp. 201-211, 1996.

[24] Farber JM, PI Peterkin. "Listeria monocytogenes, a food-borne pathogen". Microbiol. Rev, vol. 55, pp. 476-511, 1991.

[25] GM Garrity, Bell JA, TG Lilburn. Taxonomic outline of the prokaryotes. Bergey's manual of systematic bacteriology. 2nd ed. New York: Springer, 2004.

[26] Graves LM, LO Helsel, Steigerwalt AG, Morey RE, MI Daneshvar, et al. "Marthii Listeria sp. nov., isolated from the natural environment, Finger Lakes National Forest". Int J Syst Evol Microbiol, vol. 60, pp. 1280-1288, 2010.

[27] Leclercq A, Clermont D, Bizet C, Grimont PA, Le Fleche-Mateos A, et al. "Listeria sp.". Int J Syst Evol Microbiol, vol. 60, pp. 2210-2214, 2010.

[28] McLauchlin J, Mitchell RT, Smerdon WJ, K. Jewell. "Listeria monocytogenes and listeriosis: areview of hazard characterization for use in microbiological risk assessment of foods". Int J Food Microbio, vol. 92, pp. 15-33, 2004.

[29] Cummins AJ, AK Fielding, J. McLauchlin ivanovii. "Listeria infection in a patient with AIDS". J Infect, vol. 28, pp. 89-91, 1994.

[30] Guillet C, Join-Lambert O, Le MA, Leclercq A, Mechai F, et al. "Lecuit Human the caused by Listeria ivanovii listeriosis". Emerg Infect Dis, vol.16, pp. 136-138, 2010.

[31] Lessing MP, Curtis GD, Bowler IC. "Ivanovii Listeria infection". J Infect, vol. 29, pp. 230-231, 1994.

[32] Perrin M, Bemer M, Delamare C. "Fatal case of Listeria innocua bacteremia". J Clin Microbiol, vol. 41, pp. 5308-5309, 2003.

[33] Rocourt J, Hof H, Schrettenbrunner A, Malinverni R, Bille J. "Acute purulent Listeria seeligeri meningitis in an immunocompetent adult". Schweiz Med Wochenschr, vol. 116, pp. 248-251,1986.

[34] Anggun. "Hubungan Usia Ibu Hamil Dengan Kejadian Abortus". http://digilib.unimus.ac.id/gdl.php?mod=browse&op=read&id=jtptunimus-gdl-anggunnurr-5598, 2009 [Accesed on 29 April 2013].

[35] Yayasan Bina Pustaka Sarwono Prawirohardjo. Bagian Kebidanan dan Kandungan. Jakarta: Balai penerbit FK UI, pp.302-312, 2008.

[36] Cuningham G, dkk. Obstetri Williams, Vol. 2. Jakarta : EGC, 2006.

[37] Wiknjosastro, H. Ilmu Kebidanan. Jakarta : Yayasan Bina Pustaka, 2002.

[38] Kaur S, Malik SV, Vaidya VM, Barbuddhe SB. "Listeria monocytogenes inspontaneous abortions in humans and its detection by multiplex PCR". J ApplMicrobiol. vol. 103, pp. 1889–1896, 2007.

[39] Hossein Jamali, Lay Ching Chai, Kwai Lin Thong. "Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media". Food Control, vol. 32, Issue I, pp. 19-24, July 2013.